(FILE 'HOME' ENTERED AT 13:24:46 ON 19 MAR 1999)

FILE 'MEDLINE, CAPLUS' ENTERED AT 13:24:52 ON 19 MAR 1999
L1 1410 S TAC PROMOTER
L2 901 DUP REM L1 (509 DUPLICATES REMOVED)
L3 24033 S SIGNAL (1W)(SEQUENCE OR PEPTIDE)
L4 79 S L2 AND L3

=> s tandem (5a) (promoter or cassette)

L5 702 TANDEM (5A) (PROMOTER OR CASSETTE)

=> s l5 and l4

L6 1 L5 AND L4

=> d bib ab 16 1

ORDERED REF 3-19-99 QP551.P696

L6 ANSWER 1 OF 1 MEDLINE
AN 92338875 MEDLINE
DN 92338875
TI High-level expression in Escherichia coli and rapid purification of phosphatidylinositol-specific phospholipase C from Bacillus cereus and Bacillus thuringiensis.
AU Koke J A; Yang M; Henner D J; Volwerk J J; Griffith O H
CS Institute of Molecular Biology, University of Oregon, Eugene 97403..
NC GM 25698 (NIGMS)
SO PROTEIN EXPRESSION AND PURIFICATION, (1991 Feb) 2 (1) 51-8.
Journal code: BJV. ISSN: 1046-5928.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199210
AB The construction of four vectors for high-level expression in Escherichia coli of the phosphatidylinositol-specific phospholipase C from Bacillus cereus or Bacillus thuringiensis is described. In all constructs the coding sequence for the mature phospholipase is precisely fused to the E. coli heat-stable enterotoxin II **signal sequence** for targeting of the protein to the periplasm. In one set of plasmids expression of the B. cereus or B. thuringiensis enzyme is under control of the E. coli alkaline phosphatase promoter, while in a second set of plasmids expression is under control of a lac-tac-tac triple **tandem promoter**. A simple and rapid procedure for complete purification of the phospholipase C overproduced in E. coli, involving isolation of the periplasmic proteins by osmotic shock followed by a single column chromatography step, is described. The largest quantity of purified enzyme, 40-60 mg per liter culture, is obtained with the plasmid expressing the B. cereus enzyme under control of the lac-tac-**tac promoter**. Lower quantities are obtained with the plasmids containing the alkaline phosphatase promoter (15-20 and 4-6 mg/liter for the B. cereus and B. thuringiensis enzymes, respectively) and with the plasmid expressing the B. thuringiensis phospholipase under control of the lac-tac-**tac promoter** (15-20 mg/liter).